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FleQ Coordinates Flagellum-Dependent and -Independent Motilities in *Pseudomonas syringae* pv. tomato DC3000

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Motility plays an essential role in bacterial fitness and colonization in the plant environment, since it favors nutrient acquisition and avoidance of toxic substances, successful competition with other microorganisms, the ability to locate the preferred hosts, access to optimal sites within them, and dispersal in the environment during the course of transmission. In this work, we have observed that the mutation of the flagellar master regulatory gene, *fleQ*, alters bacterial surface motility and biosurfactant production, uncovering a new type of motility for *Pseudomonas syringae* pv. tomato DC3000 on semisolid surfaces. We present evidence that *P. syringae* pv. tomato DC3000 moves over semisolid surfaces by using at least two different types of motility, namely, swarming, which depends on the presence of flagella and syringafactin, a biosurfactant produced by this strain, and a flagellum-independent surface spreading or sliding, which also requires syringafactin. We also show that FleQ activates flagellum synthesis and negatively regulates syringafactin production in *P. syringae* pv. tomato DC3000. Finally, it was surprising to observe that mutants lacking flagella or syringafactin were as virulent as the wild type, and only the simultaneous loss of both flagella and syringafactin impairs the ability of *P. syringae* pv. tomato DC3000 to colonize tomato host plants and cause disease.

Motility plays a pivotal role in the spreading of bacteria across surfaces and colonization, contributing to the formation of structured communities called biofilms (1). Efficient bacterial motility under diverse environmental conditions, from liquid to semisolid and solid surfaces, is achieved by flagellum-dependent swimming and swarming or flagellum-independent twitching, gliding, nonsocial gliding, and sliding (2, 3, 4). Swimming is a flagellum-driven motility observed in bacteria moving through liquids or semisolid media, such as low-percentage agar (0.2% to 0.4%). Twitching is a slow cell movement on surfaces mediated by the extension and retraction of type IV pili (5). Gliding, a surface movement extensively studied in myxobacteria, does not require flagella or pili but involves focal adhesion complexes, cell surface-associated complexes that anchor the bacterium to a substrate and might act as a motor (6). Sliding is a passive form of surface spreading by expansion that is powered by the outward pressure of bacterial growth and facilitated by compounds that reduce friction between cells and surfaces (3). Sliding or spreading by expansion has been observed in a diverse group of bacteria, such as mycobacteria, *Bacillus subtilis*, *Vibrio cholerae*, *Serratia marcescens*, *Pseudomonas aeruginosa*, or *Legionella pneumophila* (7–12), in which a strong correlation between sliding and production of surfactants has been established. Furthermore, sliding is easily mistaken for swarming motility and can occur when flagella are disrupted in bacteria that normally would swarm (7, 8, 13, 14). Swarming is a rapid and coordinated movement of bacterial populations over a surface like semisolid agar (0.5% to 1%) and depends on flagella, pili, and the presence of a water film and/or surfactants to enable motility (2–4). Bacterial biosurfactants, in addition to having a role in multiple motility mechanisms, are involved in biofilm structure and maintenance, as well as in the delivery of insoluble signals (15, 16). Additionally, some biosurfactants have been shown to exhibit membrane-disrupting and, thus, zoosporicidal or antimicrobial activities (17). Although biosurfactants include many types of molecules, the lipopeptides, composed of an oligopeptide and a lipid tail, are particularly important and well studied in *Pseudomonas* and *Bacillus* species (17).

Bacteria belonging to the genus *Pseudomonas* are ubiquitous bacteria that are able to colonize a wide range of niches, including the soil, the plant rhizosphere and phyllosphere, and animal tissues. Motility is an important trait for these processes (18–21), biofilm formation (22–24), and pathogenesis in plants (25, 26) and animals (27). Therefore, mutations affecting flagellar regulation, biogenesis, and/or modification affect the bacterial ability to move through the environment (28), display chemotaxis toward attractants (21), and form biofilms (29, 30). In particular, motility is crucial in plant-interacting bacteria; thus, nonmotile mutants of different *Pseudomonas fluorescens* strains are severely affected in root colonization (20). Flagellum-mediated motility is also an important trait for both epiphytic and pathogenic lifestyles of *Pseudomonas syringae* (31–33). Nonmotile *P. syringae* mutants are more sensitive to desiccation and UV exposure than their motile counterparts, presumably because they cannot escape those environmental stresses (31). Additionally, their ability to invade the leaf apoplast and cause disease is severely reduced (26, 31–33).

Biogenesis and assembly of the bacterial flagellum involves a combination of transcriptional, translational, and posttransla-

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tional mechanisms that have been elucidated in *P. aeruginosa* (34, 35). In this strain, flagellar assembly is regulated in a hierarchical way, with the transcriptional activator FleQ on the top of the regulatory cascade, which involves three sigma factors, the housekeeping σ^{70} and two alternative sigma factors, σ^{54} (RpoN) and σ^{28} (FliA). In this four-tiered transcription regulation hierarchy, σ^{70} directs the expression of class I genes encoding σ^{54}, producing FliA and FleQ, an NtrC family activator protein and a cognate activator of σ^{54} (34). FleQ and σ^{54} are required for the transcriptional activation of the class II genes, like *fleSR*, and more than 20 flagellar proteins (27, 36). Class III genes, including the basal body-related genes, are positively regulated by the activated response regulator FleR in concert with σ^{54} (34, 37). The transcription of class IV genes, including *fliC*, which encodes the flagellin, is dependent on the availability of free FliA following the export of the FliA-specific anti-sigma factor FlgM through the hook-basal body apparatus (34, 38). The basic details underlying flagellar assembly and motility in a broad range of bacteria are well understood, but occasionally similar genes exert different functions depending on the strain, like the *fliT* and *fleP* genes in *P. fluorescens* F113 and *P. aeruginosa* PAO1 (18).

Aside from activating motility, FleQ also regulates genes involved in attachment to surfaces and biofilm formation (39–41). In *P. fluorescens* Pf0-1, FleQ regulates genes encoding various enzymes, putative lipoproteins, regulators, and hypothetical proteins (40). Recently it has been shown that FleQ is a negative regulator of exopolysaccharide biosynthesis in *P. aeruginosa* and *P. fluorescens* SBW25, and its activity is modulated by c-di-GMP (42–45). Mutation of *fleQ* in *P. fluorescens* SBW25 is linked to the loss of flagella and swimming motility but also promotes sliding motility, which is dependent on the biosurfactant viscosin (13). *P. aeruginosa* exhibited similar behavior on swarming plates when both pili and flagella were deleted, and biosurfactant expression was suggested as a major factor regulating this sliding motility (8).

Pseudomonas syringae pv. tomato DC3000 (*P. syringae* pv. tomato DC3000) causes bacterial speck on tomato and *Arabidopsis* and represents an important model in molecular plant pathology, since it carries a large repertoire of potential virulence factors, including proteinaceous effectors that are secreted through the type III secretion system and a polyketide phytotoxin called coronatine, which structurally mimics the plant hormone jasmonate. *P. syringae* pv. tomato DC3000 produces one to five polar flagella (46, 47), but little is known about their expression and regulation. This bacterium also produces the biosurfactant syringafactin, a peptide composed of eight amino acids with six structural variants that differ in a single amino acid and the length of the carbon chain attached (3-hydroxydecanoyl or 3-hydroxydodecanoyl). It is synthesized by a nonribosomal peptide synthetase encoded by an operon composed of two genes, *syfA* and *syfB* (48). Syringafactin is essential for swarming in *P. syringae* pv. tomato DC3000 but not in *P. syringae* pv. syringae B728a, where the loss of syringafactin severely reduced swarming but did not abolish it completely (48–50).

By using mutants *fleQ* and *fliC*, lacking flagella (46, 51), and *syfA*, impaired in the production of syringafactin (48), we have observed that the *fleQ* mutation alters bacterial surface motility and biosurfactant production, uncovering a new type of motility for *P. syringae* pv. tomato DC3000 on semisolid surfaces. Consequently, we aimed to determine the role of FleQ in the regulation of flagellar and syringafactin production genes and how both flagella and biosurfactant act on *P. syringae* pv. tomato DC3000 motility and virulence in tomato plants.

TABLE 1 Bacterial strains and plasmids used

Strain or plasmid	Relevant characteristics[a]	Reference or source
P. syringae pv. tomato strains		
DC3000	Wild type, Rif^r	52
fleQ	*fleQ*::ΩKm; Rif^r Km^r	46
fliC	*fliC* (*flaA*)::miniTn*5*Cm; Rif^r Cm^r	51
pilA	*pilA*::ΩSm/Sp; Rif^r Sm^r Sp^r	53
syfA	Δ*syfA*; Rif^r	48
syfA fleQ	Δ*syfA fleQ*::ΩKm; Rif^r Km^r	This work
Plasmids		
pBBR1MCS-5	Gm^r, cloning vector	54
pBBR1MCS-5-*fleQ*	Gm^r, pBBR1MCS-5 derivative containing the *fleQ* gene of Pto DC3000	55
pHPR-QΩKm	Ap^r Km^r, pUC18 derivative containing *P. syringae* pv. tomato DC3000 *fleQ* gene interrupted by ΩKm	46

[a] Ap^r, Cm^r, Gm^r, Km^r, Rif^r, Sm^r, and Sp^r stand for resistance to ampicillin, chloramphenicol, gentamicin, kanamycin, rifampin, streptomycin, and spectinomycin, respectively.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture media. The bacterial strains used in this study are listed in Table 1. *P. syringae* pv. tomato DC3000 and mutants were routinely grown in Luria-Bertani (LB) medium (56) at 28°C. When required, the following antibiotics were added to the cultures/plates: ampicillin (100 μg/ml), chloramphenicol (30 μg/ml), gentamicin (5 μg/ml), kanamycin (50 μg/ml), rifampin (10 μg/ml), and streptomycin (25 μg/ml).

The *syfA fleQ* null mutant was constructed by gene replacement. The Ap^r Km^r suicide plasmid pHPR-QΩKm (46) was electroporated (57) into the *P. syringae* pv. tomato DC3000 *syfA* mutant (48). Transformants that acquired the inactivated gene (Km^r) were selected and, among them, we screened for the Ap (100 μg/ml)-sensitive ones, which were expected to be the result of a double-recombination event. One of the Km^r Ap^s clones was chosen and confirmed by Southern blotting to have the wild-type *fleQ* gene replaced by the mutant allele *fleQ*::ΩKm (not shown).

Motility experiments. For swimming assays, *P. syringae* pv. tomato DC3000 and mutants were grown on LB plates for 48 h, resuspended in 10 mM $MgCl_2$ and adjusted to an optical density at 660 nm (OD_{660}), of 2.0. Two-microliter aliquots were inoculated in the center of 0.3% agar LB plates and incubated for 48 h at 25°C. The diameters of the swimming halos were measured after 48 h. For surface motility (swarming/sliding) assays, the 2-μl aliquots were inoculated in the center of PAG plates (0.5% protease peptone no. 3 [Difco 212693] and 0.2% glucose with 0.5% Difco Bacto agar) and incubated at 25°C. The surface motility was observed after 24 h and the area measured using ImageJ (58). Three motility plates were used for each strain, and the experiment was repeated with independent cultures.

Microscopy assays. The Leifson flagellum staining procedure for light microscopy was carried out according to Clark (59), with some modifications. A 1.2% solution of basic fuchsine prepared in 95% ethanol and left overnight at 25°C was mixed with an equal volume of a solution of 0.75% NaCl and 1.5% tannic acid prepared in MilliQ water. The final pH of the dye was adjusted with 1 N NaOH to pH 5.0. Swarmer cells were resuspended in 10 mM $MgCl_2$, adjusted to an OD_{660} of 1.0, and fixed by adding 1 ml of 4% formaldehyde solution per ml of culture for 20 min. The

suspension then was centrifuged, washed with MilliQ water, and resuspended in 1 ml of MilliQ water. The slides were cleaned by soaking them for 24 h at room temperature in acid dichromate solution, rinsed with MilliQ water, and then air dried. A large drop of culture suspension was deposited on the center of the slide and was allowed to run down its length and then air dried. One milliliter of dye was added and kept for 40 min, and the slide was washed with tap water, air dried, mounted with merkoglass, and examined under a Zeiss Axioskop microscope. At least 100 cells were counted per experiment, which was repeated with three independent cultures.

For negative staining, the Formvar-coated nickel grids were applied directly to bacteria grown on the agar surface at the edges of the swarming colonies and left for 5 min to allow bacterial adhesion. The grids then were washed twice in water for 1 min and negatively stained with a 1% solution of potassium phosphotungstic acid for 1 min. The grids were observed under a JEOL JEM-1011 transmission electron microscope at 100 kV at the Microscopy Service of the Estación Experimental del Zaidín, Granada, Spain.

Detection of biosurfactants. Biosurfactants were detected with the atomized oil assay previously described (49). For a more uniform inoculation of plates, *P. syringae* pv. tomato DC3000 and mutants were grown on LB plates for 48 h, resuspended in water, and adjusted to an OD_{660} of 1.0. Ten-microliter aliquots ($\sim 5 \times 10^7$ cells) were pipetted onto the surface of LB plates (1.5% agar), which were incubated for 24 h at 20°C and then sprayed with a mist of mineral oil. The diameter of the visible halo of brighter oil drops was measured, and the area of the producing bacterial colony was calculated and subtracted from that of the surfactant halo to yield the adjusted halo area. Four plates were used for each strain, and the experiment was repeated with three independent cultures.

Infection assays. Seeds of *Solanum lycopersicum* cultivar Moneymaker (a *P. syringae* pv. tomato S line) were germinated and grown with 16-h/8-h light/dark cycles at 22°C/16°C day/night and 70% relative humidity in a plant growth chamber. *P. syringae* pv. tomato DC3000 strains, grown on LB plates for 48 h at 28°C, were suspended in 10 mM $MgCl_2$, and the inoculum was adjusted to 10^8 CFU/ml. For infections, individual inocula of the strains were sprayed into different plants, and the analysis of the evolution of symptoms and sampling were performed 3 h after inoculation (time zero), when the leaves were dried, and several days after inoculation (3, 6, and 10 days postinfection [dpi]) to determine bacterial populations in plants. Bacteria were recovered from the infected leaves using a 10-mm-diameter cork-borer. Five disks (3.9 cm^2) per plant were homogenized by mechanical disruption into 1 ml of 10 mM $MgCl_2$ and counted by plating serial dilutions onto LB plates with the corresponding antibiotics. The severity of symptoms was evaluated as the percentage of necrotic area per leaflet induced by the inoculated strains after 10 days of incubation. The necrotic areas were digitally analyzed using ImageJ (58) on five inoculated leaflets of three different plants.

RNA preparation and assays. For RNA isolation, cells of *P. syringae* pv. tomato DC3000 and *fleQ* strains were grown in PAG plates and incubated at 25°C for 24 h (swarming conditions) or in MMR (7 mM Na-glutamate, 55 mM mannitol, 1.31 mM K_2HPO_4, 2.2 mM KH_2PO_4, 0.61 mM $MgSO_4$, 0.34 mM $CaCl_2$, 0.022 mM $FeCl_3$, 0.85 mM NaCl, 0.818 μM biotin, 0.296 μM thiamine, and 0.209 μM calcium pantothenate) minimal medium (60) at 20°C to an OD_{660} of 1.5. Cells were harvested and washed with 0.1% Sarkosyl, and cell pellets were immediately frozen in liquid nitrogen and conserved at −80°C until RNA isolation. For reverse transcription-quantitative real-time PCR (RT-qPCR) and transcriptome sequencing (RNA-seq), RNA was isolated using TRI Reagent LS (Molecular Research Center, Cincinnati, OH) as described before (61). Residual DNA was removed with the RNase-free DNase I set (Roche).

For RT-qPCR assays, total RNA (1 μg) treated with the RNase-free DNase I set (Qiagen) was retrotranscribed to cDNA with SuperScript II reverse transcriptase (Invitrogen) using random hexamers (Roche) as primers. Quantitative real-time PCR was performed on an iCycler iQ5 (Bio-Rad, Hercules, CA, USA). Control PCRs of the RNA samples not treated with reverse transcriptase also were performed to confirm the absence of contaminating genomic DNA. The specific primer pairs used to amplify cDNA are listed in Table 2, and primer efficiency was optimal (≈100%) for all pairs (62). Relative transcript abundance was calculated using the $\Delta\Delta C_T$ method (63). *P. syringae* pv. tomato transcriptional data were normalized to the housekeeping gene *gyrA* (61). The expression of a given gene relative to that of *gyrA* was calculated as the difference in qPCR threshold cycles ($\Delta C_T = C_{T\,\text{gene of interest}} - C_{T\,gyrA}$) The expression of each gene was determined with the control treatment (wild type) as the difference between ΔC_T values ($\Delta\Delta C_T$). As one PCR cycle represents a 2-fold difference in template abundance, fold change values were calculated as $2^{-\Delta\Delta CT}$.

For RT-PCR assays, total RNA (2 μg) was used for cDNA synthesis with SuperScript II reverse transcriptase (Invitrogen) and random hexamers as primers. Controls yielding the expected amplified fragments from all of the primers pairs were obtained by using chromosomal DNA as the template, and the presence of DNA contamination was checked using the RNA samples without RT treatment.

For comparative transcriptome analysis, total RNA was isolated from *P. syringae* pv. tomato DC3000 and the *fleQ* mutant grown under 2 different conditions: from MMR liquid cultures and from swarming plates. Library generation and sequencing was performed by Macrogen Inc. (Seoul, Republic of Korea) using Illumina HiSeq2000 to produce reads of 100 bp. Reads were clipped and trimmed to remove low-quality nucleotides, Illumina adapters, and rRNAs by using SeqTrimNext (http://www.scbi.uma.es). Filtered reads were aligned to the *P. syringae* pv. tomato DC3000 reference genome (AE016853) with Bowtie (64). SAMtools (65) utilities were used to convert sam files to bam format and to sort, index, and convert them to bedgraph files. Analysis of the data was conducted in the Integrated Genome Browser graphic user interface (http://bioviz.org/igb/).

Immunoblotting. Total cell extracts were obtained from cultures incubated in LB with shaking at 25°C. Ten milliliters of cells was centrifuged for 10 min at 2,500 × *g* at 4°C, and the pellets were kept at −80°C until the lysis step. The harvested cells then were resuspended in 200 μl of phosphate-buffered saline (PBS) with protease inhibitor mixture and lysed by sonication. Total cell protein extracts were recovered from the supernatant after centrifuging for 2 min at 15,000 × *g* and 4°C. Equal amounts of whole-cell lysates were loaded onto 10% polyacrylamide gels, electrophoresed, and transferred to polyvinylidene difluoride (PVDF) membranes. Ponceau S staining of the membranes was performed as a control of loading and transfer of comparable amounts of protein. The membranes were incubated with a 1:10,000 dilution of an anti-FliA antiserum from *P. aeruginosa* (66) and then with a peroxidase-tagged secondary antibody (anti-rabbit immunoglobulin) for 1 h. The enhanced chemiluminescence (ECL; GE Healthcare) method and Personal FX equipment was used for development. Results were analyzed with Quantity One software (Bio-Rad).

Statistical analysis. Statistical comparison among different strains or conditions was performed by one-way analysis of variance (ANOVA) with *post hoc* Tukey's honestly significant difference (HSD) test using R.

Transcriptome accession number. The RNA-seq analysis was deposited in the public database ArrayExpress at EBI under the accession number E-MTAB-3779.

RESULTS

***P. syringae* pv. tomato DC3000 employs flagellum-dependent and flagellum-independent syringafactin-dependent surface motilities.** To investigate the role of flagella in *P. syringae* pv. tomato DC3000 motility, we used an *fleQ* mutant deficient in the master regulator for flagellar synthesis (46) and an *fliC* mutant impaired in flagellin production (previously called *flaA*) (51). Surface (swarming and sliding) motility assays were carried out in PAG (0.5% agar) plates to detect the ability to spread over the agar surface. As a control, we performed swimming assays in LB (0.3%

TABLE 2 Primers used in this study

Primer name	Sequence (5′→3′)	Use
flhA+	TATTCGCAGCATTGCAGAGG	*flhA-flhF* intergenic region amplification
flhF−	GCATCGAGTCAAAGGCACGC	*flhA-flhF* intergenic region amplification
flhF+	CAGTATGGACAGTTTCCGCATCGGC	*flhF-fleN* intergenic region amplification
fleN−	TTGTCGCCAATCTCGCTGAACGCC	*flhF-fleN* intergenic region amplification
fleN+RT	AGGCGTTCAGCGAGATTGGCGACA	*fleN-fliA* intergenic region amplification
fliA−RT	ACGCTCGCGCTCTGGCAAATTGG	*fleN-fliA* intergenic region amplification
fleQ-F	GCGAGAGGTTCTGTGCGTGCTGGTC	qRT-PCR expression of *fleQ*
fleQ-R	GCAGCTCGACGGAAGAGTTTTCGCTCA	
fleS-F	CGCCAGTCATCTGACCGAGCAGGAA	qRT-PCR expression of *fleS*
fleS-R	GGGCGTTAGGCGGTCCGTCAGC	
fleN-F	AGGCGTTCAGCGAGATTGGCGA	qRT-PCR expression of *fleN*
fleN-R	TCGCACACCACCAGCAGCACTTCCT	
fliA-F	CGAGGACGGCGCAAGCGGTTC	qRT-PCR expression of *fliA*
fliA-R	ACGCTCGCGCTCTGGCAAATTGG	
flgM-F	CACGTACCGCAGCCAGCAAAGACACC	qRT-PCR expression of *flgM*
flgM-R	TAACGGTAGGCAGGTCGGTCAGCTTGTCC	
flgF-F	TCGACCAATGGCTTCATGCGTGACCTT	qRT-PCR expression of *flgF*
flgF-R	TCGGGAGTCTGCACGGCAATCCAG	
flhA-F	CGTCGTGCTGCTGGTTTGCGTCTAT	qRT-PCR expression of *flhA*
flhA-R	GCATGACCGTCCTGACCGTGAAGC	
flhF-F	GCCGCCACGCCCGTTTGC	qRT-PCR expression of *flhF*
flhF-R	GCTGCGGACGGCTGCCTTGT	
fliC-F	AAGGCGCACTGCAAGAGTCGACCAAC	qRT-PCR expression of *fliC*
fliC-R	GGTGCTGGCGGAACCGTCAAGC	
syfA-F	CGCACGACGCAACGCAAGGAATGG	qRT-PCR expression of *syfA*
syfA-R	ACGCACGGCACTTACCTGGGCAAA	
syfR-F	CCGCTGGTCGAACATCACGCACAA	qRT-PCR expression of *syfR*
syfR-R	AGCCACGGTTCCGCCTGTCAA	
gyrA-F	GGCAAGGTCACCCGCTTCAAGGAAT	qRT-PCR expression of *gyrA*
gyrA-R	GACCGCCACGCTTGTACTCAGGGAAC	

agar) plates and detected the cells moving through the agar (Fig. 1). We observed that the *fliC* and *fleQ* mutants remained immobile in swimming plates (Fig. 1A). On PAG, the surface motility of the *fliC* mutant was severely affected, whereas the *fleQ* mutant exhibited altered surface spreading on semisolid agar (Fig. 1B). To confirm that the phenotypic changes were due to the loss of FleQ, the mutant was complemented with an intact copy of the *fleQ* gene in a plasmid. The complemented strain produced flagella, regained the ability to swim through agar, and exhibited a swarming motility phenotype similar to that of the wild type (see Fig. S1 in the supplemental material).

Swarming is powered by flagella but also is influenced by other surface structures, like type IV pili (TFP) (2–4). To investigate the role TFP in *P. syringae* pv. tomato DC3000 motility, a *pilA* knockout mutant (53) lacking those organelles was tested in motility assays (Fig. 1). We observed that the *pilA* mutant exhibited a 20% reduction on swimming motility (Fig. 1A), but its swarming motility increased 30% compared to that of the wild type (Fig. 1B). Therefore, TFP are dispensable for swimming (Fig. 1A), and they seem to restrict swarming in the wild-type strain (Fig. 1B), as has been observed in *P. aeruginosa* (8, 67, 68).

The surface motility exhibited by the *P. syringae* pv. tomato DC3000 *fleQ* mutant is similar to that reported for different bacteria after blocking flagellum production (7, 8, 13, 14) and could be considered sliding, a passive form of surface spreading which relies on bacterial growth and biosurfactant production (3). As has been mentioned before, *P. syringae* pv. tomato DC3000 produces the biosurfactant syringafactin, and a mutant blocked in syringafactin production (*syfA*) could swarm no longer (48, 49). Also, under our experimental conditions (PAG–0.5% agar plates), the swarming of the *P. syringae* pv. tomato DC3000 *syfA* mutant was completely inhibited (Fig. 1). However, its swimming motility (in LB–0.3% agar plates) was similar to that of the wild type (Fig. 1).

To assess whether all of those strains produced flagella, a flagellar stain for light microscopy was used in swarmer cells (59), observing that *P. syringae* pv. tomato DC3000 produced 2 to 5 polar flagella, more than it did in liquid medium-grown cells (46, 47). The *fleQ* and *fliC* mutants were devoid of these organelles, as expected; however, the *syfA* mutant produced flagella similar in number and morphology to those of the wild type (Fig. 1C). These results confirm that the flagellum is essential for swimming motility in *P. syringae* pv. tomato DC3000 and plays an important role in its surface motility, whereas TFP seem to have an accessory role. On the other hand, syringafactin is not required for swimming but is absolutely indispensable for swarming, at least under the conditions tested.

FleQ is the master regulator of flagellar genes in *P. syringae* pv. tomato DC3000. In *P. aeruginosa*, the master regulator FleQ belongs to the top tier of the flagellar cascade and is required to activate all other flagellar genes, with the exception of *fliA* (27, 34, 36). To advance our knowledge of the flagellar regulatory cascade in *P. syringae* pv. tomato DC3000, which has not been characterized so far, and to establish the role of FleQ in this strain, we analyzed the effect of the *fleQ* deletion on transcript levels by RNA-seq. We compared the wild-type strain and the *fleQ* mutant

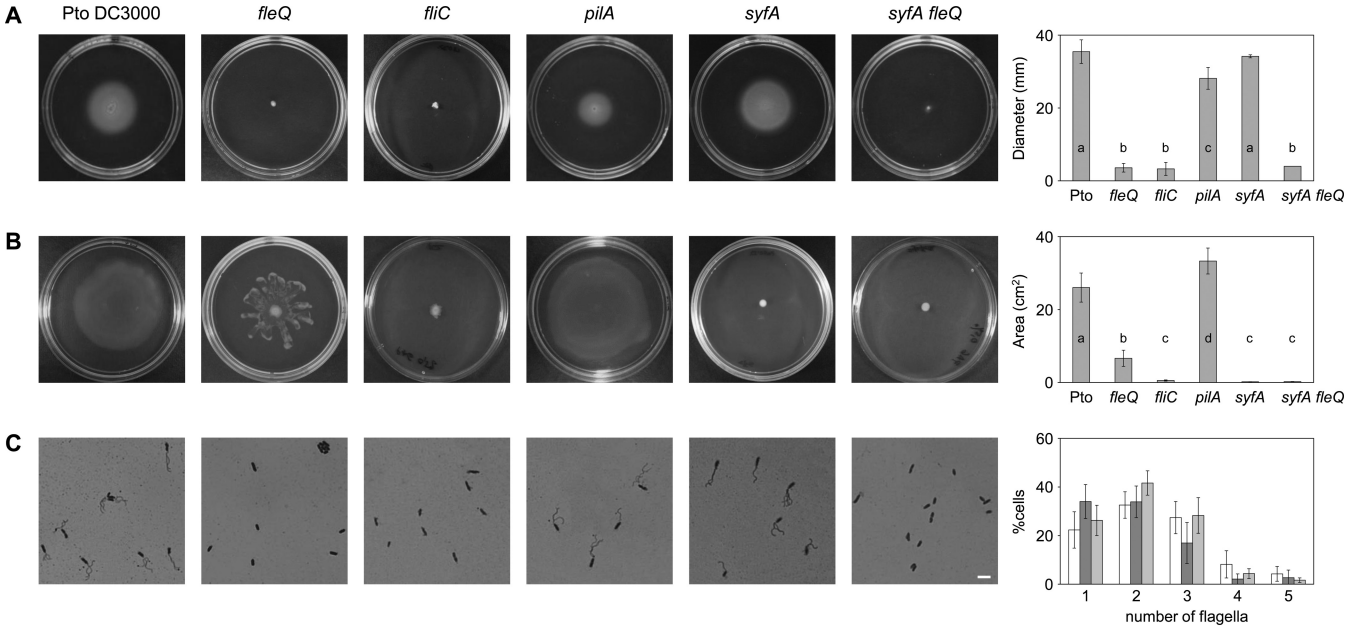

FIG 1 Motility of *P. syringae* pv. tomato DC3000 and its mutants. (A) Swimming motility assays were performed with the wild type and the indicated mutants. Pictures were taken 48 h after inoculation and the swimming halo diameter measured. The graphic shows the means and standard deviations from four experiments with three replicates; a, b, and c denote ANOVA categories with significant differences ($P < 0.01$). (B) Surface motility assays with the wild type and the indicated mutants 24 h after inoculation. The areas of motility were calculated with ImageJ and the means and standard deviations from five experiments with three replicates plotted; a, b, c, and d denote ANOVA categories with significant differences ($P < 0.01$). (C) Visualization of flagellar abundance in *P. syringae* pv. tomato DC3000 strains taken from PAG plates. Light microphotographs of cells from PAG plates stained with the Leifson method. The scale bar (white) represents 5 μm. The graphic shows the means and standard deviations of the percentages of *P. syringae* pv. tomato DC3000 (white), *pilA* (dark gray), and *syfA* (light gray) cells with 1 to 5 flagella. The *fleQ*, *fliC*, and *syfA fleQ* mutants did not produce any flagella. At least 100 cells were counted per experiment, which was repeated with four independent cultures. No significant differences in flagellum numbers were detected by one-way ANOVA between the wild type and the *syfA* mutant.

in swarming and in liquid culture-grown cells as a control, observing that the majority of the flagellar genes were expressed similarly under both conditions (Fig. 2A). Also, several genes were strongly downregulated in the *fleQ* mutant, like *flgBCDE*, *flgFGHIJKL*, *fliC*, *flaG*, *fliD*, *fliS*, *fliT*, *fleSR*, *fliEFGHIJ*, *cheY-2*, *cheZ*, and *cheA-2*, among others. These results confirm the role of *P. syringae* pv. tomato DC3000 FleQ as a master regulator of the flagellar genes, like in other pseudomonads (18, 27, 34, 36, 41). In order to validate the RNA-seq data, we analyzed the expression of several motility genes presumably belonging to different classes of the flagellar gene hierarchy (*fleQ*, *fleS*, *flhA*, *flhF*, *fleN*, *fliA*, *flgF*, *flgM*, and *fliC*) under swarming conditions (Fig. 2B). The RT-qPCR analysis carried out revealed that the expression of *fleS* and *fliC* is strictly dependent on FleQ, similar to what occurs in *P. aeruginosa*. However, transcription of *flgF*, *flhA*, *flhF*, *fleN*, *fliA*, and *flgM* seem to be regulated only to some extent by FleQ, unlike in *P. aeruginosa*, where *fliA* is a class I gene which expresses independently of FleQ, *flgF*, *flhA*, *flhF*, and *fleN* are strictly dependent on the activation by FleQ, and *flgM* also is positively regulated by FliA (34, 36).

Since the expression of *fliA* and some adjacent genes (*flhA*, *flhF*, and *fleN*) in *P. syringae* pv. tomato DC3000 was different from that of *P. aeruginosa*, we decided to ascertain the transcriptional organization of the *flhA-fliA* region and its expression in both the wild type and the *fleQ* mutant. Primers for coamplification of adjacent genes in the *flhA-fliA* region were designed, and total RNA from cells of *P. syringae* pv. tomato DC3000 and the *fleQ* mutant grown on PAG plates (swarming conditions) was subjected to RT-PCR (Fig. 3A). The results show that the *flhA-flhF*, *flhF-fleN*, and *fleN-fliA* primer pairs each yielded an RT-PCR product, indicating that in *P. syringae* pv. tomato DC3000, *flhA-flhF-fleN-fliA* is transcribed as a tetracistronic operon (Fig. 3B), which is different from *P. aeruginosa*, where *flhA* and *fliA* are transcribed as monocistronic operons and *flhF* and *fleN* are transcribed as a bicistronic operon (34). In fact, the *P. syringae* pv. tomato DC3000 *flhA* transcription start site was previously determined (69, 70), and we have identified a putative σ^{54} promoter upstream of *flhA* and a FleQ binding site which, similar to that in *P. aeruginosa* (36, 42), is located within the leader region (+17/+22; see Fig. S2 in the supplemental material). Furthermore, *flhA*, *flhF*, *fleN*, and *fliA* also were cotranscribed in the *P. syringae* pv. tomato DC3000 *fleQ* mutant (Fig. 2A and 3B; also see Fig. S3 for a detailed view). Therefore, *flhA-flhF-fleN-fliA* genes transcribe as an operon not only in the presence of FleQ but also in its absence, albeit at lower levels, providing basal concentrations of FlhA, FlhF, FleN, and FliA proteins. To corroborate this hypothesis, immunoblots of total cell extracts from the wild type and the *fleQ* mutant were carried out, showing that the production of FliA was maximal in the wild type and significantly diminished in the *fleQ*-lacking strain (Fig. 3C). Therefore, not only *fliA* transcription but also FliA production are reduced in the *fleQ* mutant, confirming that, in *P. syringae* pv. tomato DC3000, FliA synthesis is mostly dependent on the master regulator (Fig. 2 and 3). The results presented here corroborate that flagellar biosynthesis is differently regulated in diverse *Pseudomonas* strains (71).

FleQ negatively regulates syringafactin production in *P. syringae* pv. tomato DC3000. Although the flagellum plays an important role in *P. syringae* pv. tomato DC3000 surface motility,

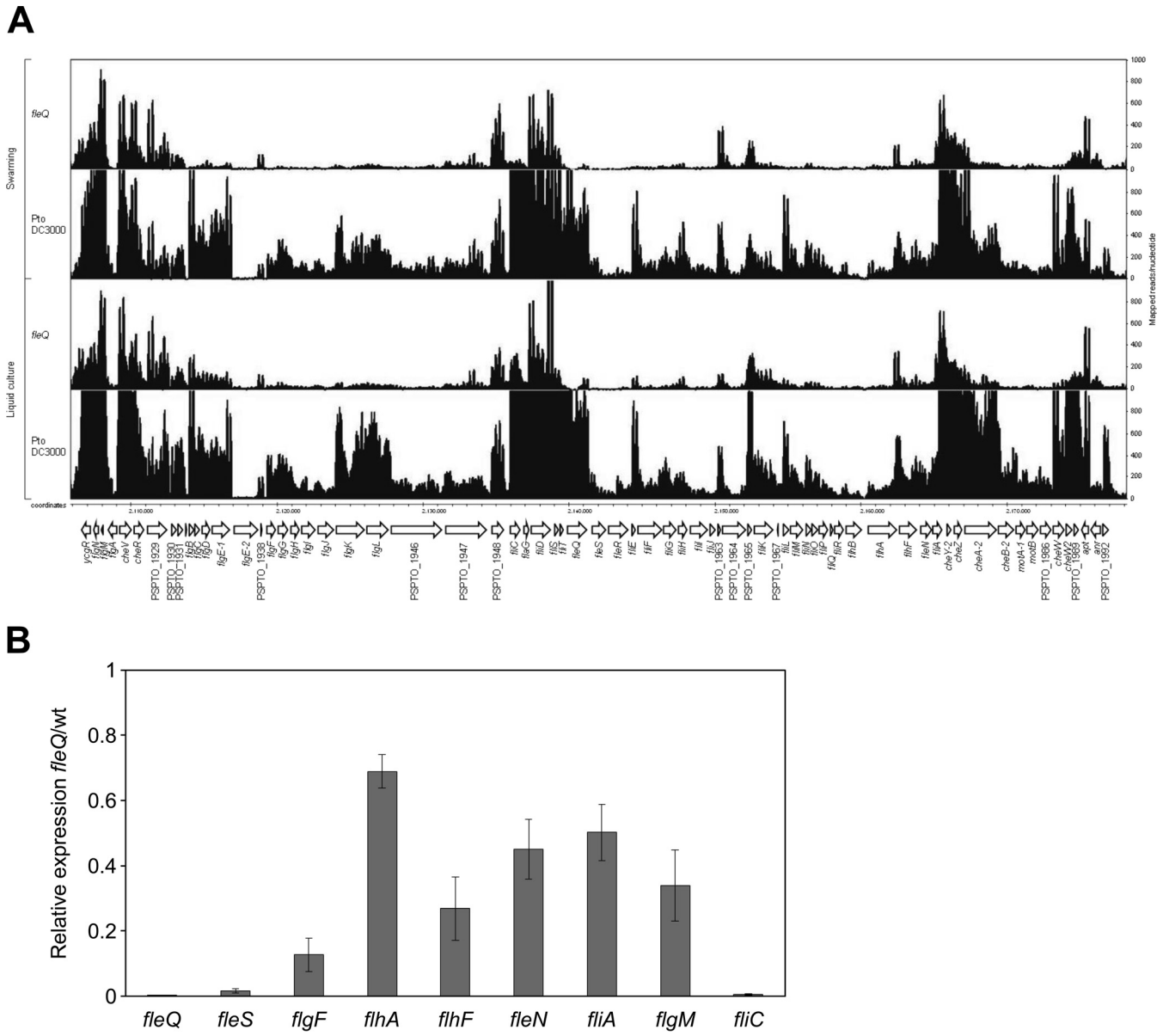

FIG 2 Expression analysis of flagellar genes in *P. syringae* pv. tomato DC3000. (A) RNA mapped read distribution of flagellum-encoding genes in *P. syringae* pv. tomato DC3000 and *fleQ*. RNA was obtained from *P. syringae* pv. tomato DC3000 and *fleQ* grown under swarming conditions and liquid cultures (MMR at 20°C). Drawings are images from IGB software showing reads in several genes belonging to the flagellar cascade. Genomic coordinates denote the position in kilobases of the *P. syringae* pv. tomato DC3000 genome, and annotated ORFs are shown as arrows. The scale (counts) indicates the number of mapped reads per nucleotide position. (B) Expression of several flagellar genes determined by RT-qPCR. Total RNAs and cell extracts were obtained from bacteria grown in PAG (0.5% agar) plates for 24 h at 25°C. RT-qPCR expression values of the genes in the *fleQ* mutant were normalized with the housekeeping gene *gyrA*. The relative expression of flagellar genes was calculated as the fold change between the *fleQ* mutant and the wild-type strain (which was set to 1.0). Results shown are the means and standard deviations from three independent biological experiments with three technical replicates each. In all cases, differences with respect to the wild type were significant as determined by one-way ANOVA ($P < 0.01$).

other mechanisms may facilitate its displacement over surfaces, as suggested by the movement exhibited by the *fleQ* mutant. In this regard, a *P. fluorescens* SBW25 *fleQ* mutant exhibited a spreading surface motility that required the expression of *viscB* and *viscC*, which encode a nonribosomal peptide synthetase involved in the production of the lipopeptide surfactant viscosin (13). Likewise, we hypothesized that the inactivation of *fleQ* in *P. syringae* pv. tomato DC3000 leads to an increase in the production of surfactant(s). To check this possibility, we assessed the production of surfactants by the atomized oil assay (49). The amount of surfactants produced by the *fleQ* mutant was increased by 40% compared to that produced by the wild-type strain or the *fliC* mutant (Fig. 4A), suggesting this is the cause of its altered spreading motility. To test this hypothesis, we constructed an *syfA fleQ* double mutant, observing that this mutant did not exhibit swimming or surface motility and lacked flagella (Fig. 1). We were unable to generate a complemented mutant for syringafactin production; instead, we carried out RNA-seq to investigate the role of FleQ in

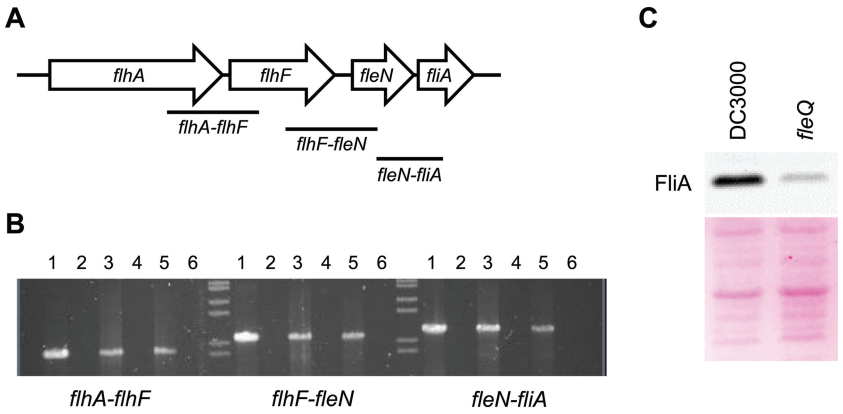

FIG 3 Expression of the *flhA-fliA* region in *P. syringae* pv. tomato DC3000. (A) Map of the *flhA-fliA* region. The labeled bars indicate the expected fragments amplified with each primer pair (Table 2), with each of them partially covering two adjacent genes. (B) Results from RT-PCR amplification of the *flhA-fliA* region. Total RNA was obtained from *P. syringae* pv. tomato DC3000 and the *fleQ* mutant grown in swarming conditions. Lanes 1 and 2, positive (chromosomal DNA as the template yielding the expected amplified fragments from all of the primer pairs) and negative (water) controls; lanes 3 and 5, 2 μg of RNA samples from the wild type and the *fleQ* mutant, respectively, with RT treatment; lanes 4 and 6, the presence of DNA contamination was checked using 2 μg of RNA samples of the wild type and the *fleQ* mutant, respectively, without RT treatment. (C) Production of FliA by *P. syringae* pv. tomato DC3000 and *fleQ*. Protein was detected using an anti-FliA antibody from *P. aeruginosa* (66). FliA appeared on membranes as a band of approximately 29 kDa in apparent size. Ponceau staining of the membrane was used as a loading and transfer control (bottom).

syringafactin synthesis at the transcriptional level, noticing that the expression of *syfR* and *syfABCD* genes is upregulated in the *fleQ* mutant (Fig. 4B). To corroborate these results, we carried out RT-qPCR assays and observed that *syfR* and *syfA* mRNA levels were increased (2.5- ± 0.1-fold and 2.4- ± 0.3-fold, respectively) in the *fleQ* mutant (Fig. 4C). *syfR* (PSPTO_2828) encodes a transcriptional regulator of the LuxR family which seems to function as an activator for the *syfABCD* genes, since an insertion in *syfR* resulted in the loss of swarming motility, droplet collapse, and syringafactin accumulation (48). The increased expression of *syfR* and *syfA* in the absence of FleQ suggests that the flagellar master regulator is negatively regulating *syfR* and, consequently, *syfAB*(*CD*) expression and syringafactin production in the wild-type strain.

Role of *P. syringae* pv. tomato DC3000 flagellar and nonflagellar motility in virulence. Once we established the role of flagella and syringafactin and their regulation by FleQ in the different movements exhibited by *P. syringae* pv. tomato DC3000, we next studied whether the flagella and syringafactin contributed to the pathogenic process of *P. syringae* pv. tomato DC3000. For this, we compared the abilities of the wild type and the *fleQ*, *syfA*, and *syfA fleQ* mutant strains to survive and multiply in tomato leaf tissues by monitoring bacterial populations and development of disease symptoms for 10 days after inoculation by spray. We also used the *fliC* mutant, since this strain has a functional FleQ but lacks flagella due to the *fliC*/flagellin deficiency. The disease symptoms caused by the *fliC*, *fleQ*, and *syfA* single mutants were indistinguishable from those caused by the wild-type strain: small water-soaked lesions surrounded by large regions of chlorosis that appeared 2 to 3 days after inoculation. These spots soon turned brown, and the tissue surrounding the spots became yellow. However, the symptoms caused by the *syfA fleQ* double mutant were far less abundant (Fig. 5A).

Before the plant experiments, all of the mutants and the wild type were tested for growth in different laboratory media and showed similar growth curves (not shown). However, their behavior in tomato leaves varied, although the starting populations of the five strains were similar (Fig. 5B). The *syfA fleQ* mutant was significantly growth impaired *in planta*, as evidenced by the small population sizes at all time points. The wild type and the *fleQ* mutant behaved similarly: their populations reached a maximum at 3 dpi (1.8×10^7 to 2.1×10^7 CFU/cm^2) and after that decreased to 1.5×10^6 to 1.9×10^6 CFU/cm^2 at 10 dpi. The *fliC* and *syfA* mutants behaved similarly and exhibited an intermediate phenotype between that of the *syfA fleQ* mutant and that of the wild type and *fleQ*: their populations reached a maximum at 3 dpi (4.4×10^6 to 5.4×10^6 CFU/cm^2) and after that decreased to 1.0×10^5 to 1.9×10^5 CFU/cm^2. These results indicate that only the combined loss of *fleQ* and *syfA* causes a significant decrease in the ability of *P. syringae* pv. tomato DC3000 to colonize tomato plants and cause disease. However, mutants deficient in flagella (*fliC*) or syringafactin (*syfA*) were not able to reach population densities as high as those of the wild type or the *fleQ* mutant but caused similar symptoms. Interestingly, the *fleQ* mutant, which also lacks flagella, behaved like the wild type in both growth and symptom development.

DISCUSSION

Bacteria can move through their environment in multiple ways. Several genera of bacteria, including pseudomonads, exhibit swarming, a complex form of motility on semisolid surfaces which is absolutely dependent on flagella but also influenced by specific environmental signals, the presence of other surface structures, and the production of surfactants (8, 13, 72). In this work, we present evidence that *P. syringae* pv. tomato DC3000 is able to display different types of motility. The predominant movement in liquid or semisolid media is swimming, which requires the presence of flagella, whereas the biosurfactant syringafactin is dispensable (Fig. 1A). In contrast, both flagella and syringafactin are required for swarming motility (Fig. 1B). We also show that *P. syringae* pv. tomato DC3000 displays an alternative form of surface motility that is unmasked upon inactivation of the regulator FleQ. This type of motility is consistent with the notion that bacterial translocation is facilitated by both propulsive forces and

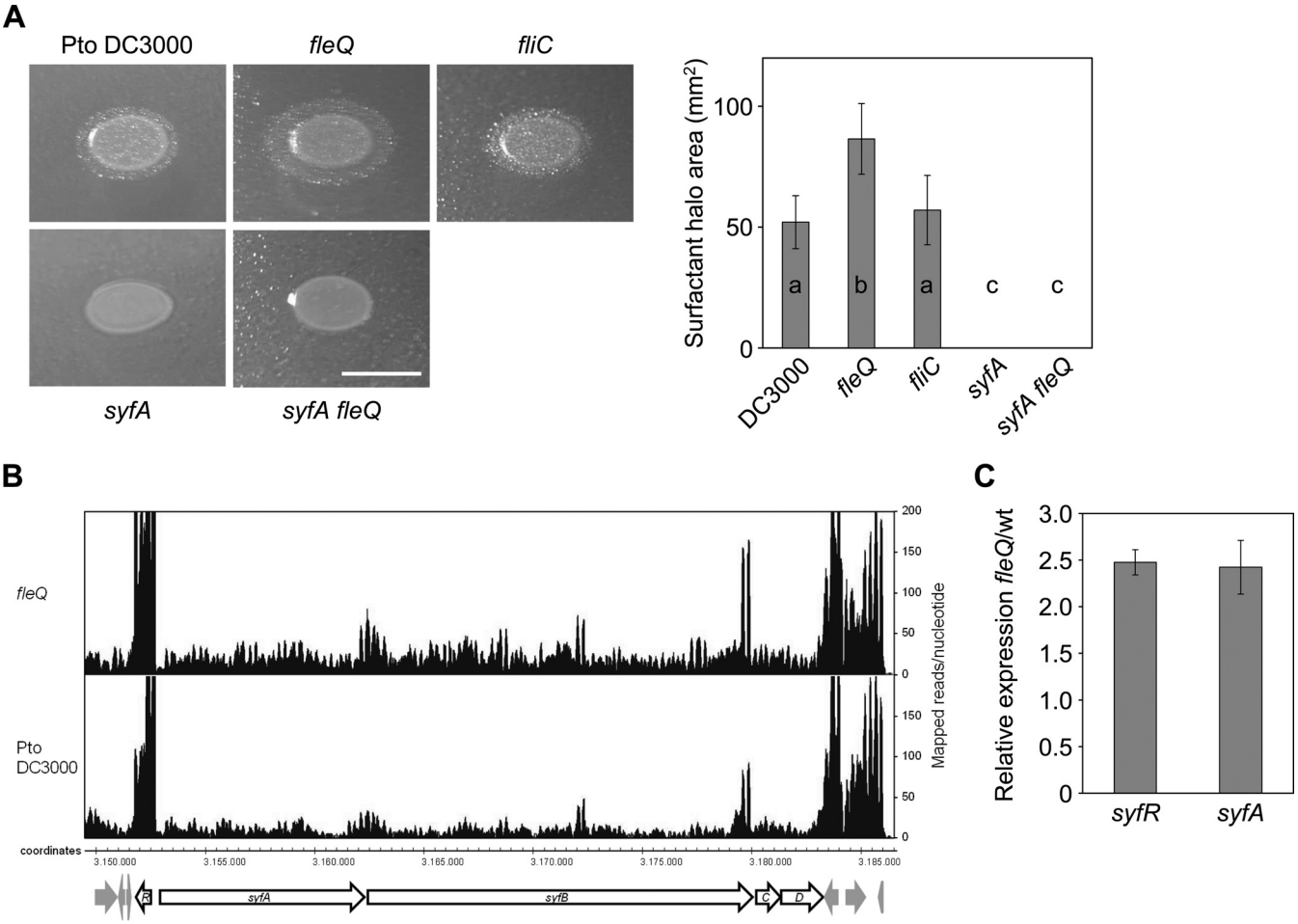

FIG 4 Syringafactin expression and production in *P. syringae* pv. tomato DC3000 and its mutants. (A) Comparison of surfactant-induced halos around bacterial colonies grown on LB (1.5% agar) for 24 h at 20°C and visualized by the atomized oil assay. The biosurfactant areas were calculated and the means and standard deviations of three experiments with four replicates plotted; a, b, and c denote ANOVA categories with significant differences ($P < 0.01$). The scale bar (white) represents 1 cm. (B) *P. syringae* pv. tomato DC3000 and the *fleQ* mutant mapped read distribution in the *syf* region under swarming conditions. Drawings are images from IGB software showing reads in *syfR*, *syfA*, *syfB*, *syfC*, and *syfD*. Genomic coordinates denote the position in kilobases of the *P. syringae* pv. tomato DC3000 genome, and annotated ORFs are shown as arrows. The scale (counts) indicates the number of mapped reads per nucleotide position. (C) Expression analysis of *syfA* and *syfR* in *P. syringae* pv. tomato DC3000 and *fleQ* determined by RT-qPCR. Total RNAs and cells extracts were obtained from bacteria grown in PAG (0.5% agar) plates for 24 h at 25°C. RT-qPCR expression values of the genes in the *fleQ* mutant were normalized with the housekeeping gene *gyrA*. The relative expression of flagellar genes was calculated as the fold change between the *fleQ* mutant and the wild-type strain (set to 1.0). Results shown are the means and standard deviations from three independent biological experiments with three technical replicates each. In all cases, differences with respect to the wild type were significant as determined by one-way ANOVA ($P < 0.01$).

those that reduce friction between bacterium and substrate (3, 8, 73, 74). In fact, the absence of flagella is not sufficient to enable sliding motility, and an *fliC* mutant is not able to spread on plates (Fig. 1B). Furthermore, not only swarming, as it was shown before (48, 49), but also sliding are strictly dependent on the production of the biosurfactant syringafactin, since both an *syfA* mutant and a double *syfA fleQ* mutant are not able to spread on plates (Fig. 1B). We should mention that this is a consistent phenotype exhibited under different conditions, KB (0.4 and 0.5% agar) (48, 72) and PAG plates (0.5% agar) (this work), suggesting that both flagellum-dependent motility and flagellum-independent, syringafactin-dependent motility are in operation on the agar plates. The fact that syringafactin production is crucial for both swarming and sliding in *P. syringae* pv. tomato DC3000 seems to be unique among *P. syringae* strains, since *P. syringae* pv. syringae B728a syringafactin mutants exhibited greatly reduced surfactant halos, drop collapse ability, and swarming, but only the lack of 3-(3-hydroxyalkanoyloxy) alkanoic acid (HAA) production in a syringafactin-deficient strain completely abolished surface motility (72).

An *fleQ* mutant of *P. syringae* pv. syringae B728a previously was deficient in surfactant (HAA and syringafactin) production (72). However, we have shown the opposite: the *P. syringae* pv. tomato DC3000 *fleQ* mutant overproduced syringafactin (Fig. 4A); furthermore, its sliding motility on semisolid surfaces is similar to that of the *P. fluorescens* SBW25 *fleQ* mutant, which was shown to require the surfactant viscosin (13). This suggests that the control of biosurfactant production by FleQ is a common feature in pseudomonads. Particularly in *P. syringae* pv. tomato DC3000, syringafactin production is enhanced under conditions in which *fleQ* is downregulated, assuring bacterial motility over surfaces. It remains to be determined whether the effect of FleQ on

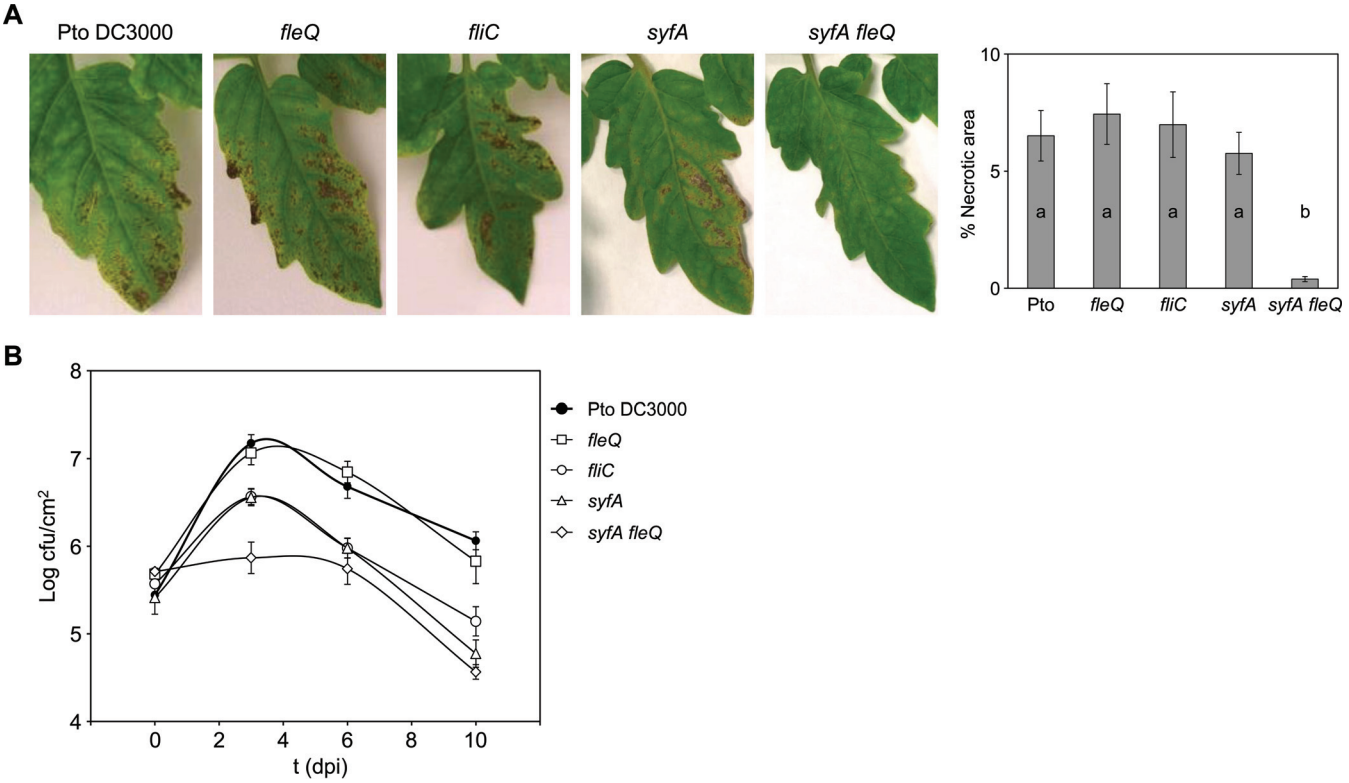

FIG 5 Bacterial growth and symptom development on tomato leaves. (A) Development of symptoms induced on tomato leaves 10 days after inoculation with *P. syringae* pv. tomato DC3000 and different mutants at 10^8 CFU/ml by spray. The severity of symptoms was evaluated as the percentage of necrotic area per leaflet induced by the inoculated strains. The graphic shows the means and standard errors from three different experiments (with five leaflets analyzed in each of them); a and b denote ANOVA categories with significant differences ($P < 0.01$). (B) Time course of growth of *P. syringae* pv. tomato DC3000 and the *fleQ*, *fliC*, *syfA*, and *syfA fleQ* mutants in the primary leaves of tomato plants. CFU were quantified at 0, 3, 6, and 10 dpi with approximately 10^8 CFU/ml by spray. Data represent the averages from three experiments with their standard errors. At 3 days, differences between the wild type and the *fleQ* mutant and between the *fliC* and *syfA* mutant populations were not significant. At 6 and 10 dpi, *fliC*, *syfA*, and *syfA fleQ* mutant populations were significantly different from those of the wild type and the *fleQ* mutant as determined by one-way ANOVA ($P < 0.01$); differences between the wild type and the *fleQ* mutant or among the *fliC*, *syfA*, and *syfA fleQ* mutant populations were not significant.

syringafactin production is direct (transcriptional regulation of the *syf* genes) or, most likely, mediated by additional factors/regulators, since no conserved sites for FleQ binding were detected in the *syfR-syfA* intergenic region.

We also have established the role of FleQ as the master regulator of flagellar genes in *P. syringae* pv. tomato DC3000 and spotted some differences in the regulation of flagellar biosynthesis compared to that of other *Pseudomonas* organisms. We have demonstrated that *flhA*, *flhF*, *fleN*, and *fliA* transcribe as an operon in the presence of FleQ but also in its absence, although at low levels (Fig. 2 and 3). This situation is different from that of *P. aeruginosa*, where FleQ regulates the expression of the majority of flagellar genes, with the exception of *fliA*, grouping *fleQ* and *fliA* as class I genes (34). Therefore, we propose that *fliA* should be considered a class II gene in *P. syringae* pv. tomato DC3000, like *flhA*, *flhF*, and *fleN*, because its expression is partially dependent on the master regulator. Moreover, the lower levels of FliA detected in the *fleQ* mutant suggest that FleQ controls expression of *fliA* not only at the transcriptional level but also posttranscriptionally. In *Escherichia coli*, FliA degradation increases when its anti-sigma factor FlgM is not available due to secretion or mutation (75). In the *P. syringae* pv. tomato DC3000 *fleQ* mutant, *flgM* expression also is reduced (Fig. 2); therefore, the FliA:FlgM stoichiometry would be altered and FliA degraded. Nevertheless, the low expression of the *flhA-flhF-fleN-fliA* operon in the absence of FleQ provides basal levels of FliA (Fig. 3) and most likely of FlhA, FlhF, and FleN proteins. Since *P. syringae* pv. tomato DC3000 produces 1 to 5 polar flagella, whereas *P. aeruginosa* possesses a single polar flagellum, basal levels of those proteins may be needed for rapid flagellum assembly. Also, those proteins may have additional functions in virulence factor secretion, cytotoxicity, or proper cell division, as has been shown in *Bacillus thuringiensis* (76), *Xanthomonas campestris* pv. campestris (77), *Yersinia enterocolitica* (78), and *Campylobacter jejuni* (79).

In summary, we have shown that FleQ activates flagellum synthesis and negatively regulates syringafactin production in *P. syringae* pv. tomato DC3000, thereby controlling not only swimming and swarming but also the sliding motility in this strain.

Bacterial flagella are considered an important virulence factor in *P. aeruginosa*, *C. jejuni*, and *Salmonella enterica* serovar Typhimurium mainly because of their role in motility and chemotaxis, facilitating the pathogen-host interactions and promoting colonization, penetration, and invasion of host tissues (80–82). The importance of flagella in plant colonization is a controversial subject with contradictory results. Some researchers showed that flagella were essential for *P. fluorescens* root colonization (20) or move-

ment onto the root surface and attachment (83), whereas others suggested that passive movement with water flow played a more important role than motility (84). *S. enterica* and enterohemorrhagic *E. coli* require flagella for systemic spread from root to shoot of *Arabidopsis thaliana* (85), whereas flagella are essential to colonize certain plants only for some *Listeria monocytogenes* strains (86). Here, we have shown that mutation of *fliC* in *P. syringae* pv. tomato DC3000 did not abolish its ability to reach the apoplast and cause infection, although its populations were smaller than those of the wild type (Fig. 5), as was observed before (87). This is different from other *Pseudomonas* strains (88) and pathovars of *P. syringae* (30, 33, 89) and suggests that *P. syringae* pv. tomato DC3000 uses alternative forms of motility that are not flagellum dependent for the colonization of tomato leaves. It has been shown that flagellum-based swarming is insufficient for movement over dry surfaces, where biosurfactants may acquire a more important role by promoting water retention and/or surface spreading (8, 13, 50, 90). In this sense, the *P. syringae* pv. tomato *fliC* mutant is able to produce syringafactin at levels comparable to those of the wild type (Fig. 4).

On the other hand, we also have shown that the *syfA* mutant provoked symptoms similar to those of the wild type, although it was not able to multiply at the same level (Fig. 5). Therefore, the absence of syringafactin did not prevent *P. syringae* pv. tomato DC3000 from reaching the apoplast and causing infection, which indicates that this surfactant is not phytotoxic *per se* and is not essential for *P. syringae* pv. tomato DC3000 colonization of tomato leaves when flagella are present and bacteria can swim. This is different from other phytopathogenic *Pseudomonas* species, like *P. cichorii*, whose biosurfactants (cichofactins) significantly contribute to lettuce midrib invasion (91). Moreover, in the *fleQ* mutant, the overproduction of syringafactin seems to counteract the absence of flagella (compared to the *fliC* and the *syfA fleQ* mutants), increasing bacterial survival and/or growth *in planta*. Therefore, colonization and symptom development in tomato are seriously compromised only when both flagella and syringafactin are missing, but neither flagella nor syringafactin on their own are essential for leaf surface migration, colonization, and virulence in *P. syringae* pv. tomato DC3000, although they may have a role in leaf invasion and/or survival given that the mutant population levels remain lower than those of the wild type (Fig. 5). Thus, the production of both flagella and syringafactin in *P. syringae* pv. tomato DC3000 seems to play a role in leaf surface dissemination and also in apoplast colonization, since they probably facilitate access to nutrients and bacterial survival. Since the overproduction of syringafactin compensates for the absence of flagella in *P. syringae* pv. tomato DC3000 colonization of tomato leaves, we propose that sliding movement and positive chemotaxis toward stomata are the driving forces allowing *P. syringae* pv. tomato DC3000 to rapidly reach stomata and enter the apoplast, similar to the sliding motility linked to bacterial cell division of rhizobia within legume root infection threads (92, 93). Moreover, *P. syringae* pv. tomato DC3000 may have developed this alternative form of sliding motility to colonize plant surfaces under conditions in which the production and/or activity of flagella is not optimal or flagellar expression results are counterproductive, since flagellin triggers host immune responses (94) with FleQ as the regulator that coordinates the switching between flagellum-dependent and flagellum-independent, syringafactin-dependent motilities.

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